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The occurrence of *Rutstroemia coracina* on *Quercus ilex* leaves in Spain

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Abstract— The authors report new findings of an apparently rare fungus, *Rutstroemia coracina* (*Rutstroemiaceae*). The species grew on old fallen leaves of *Quercus ilex* subsp. *ballota* at different localities in Guadalajara and Madrid provinces (center of Spain) where it was first collected in 1983 and subsequent years. The apothecia are described and illustrated in detail. Microscopic studies, including microtome sections, show a clear gel layer in the ectal excipulum, which allows assignment in the genus *Rutstroemia*. Since the type material deposited in PC is so scanty and deteriorated that any future revision will be impossible, an epitype is selected here.

Key words— *Ascomycota*, *Helotiales*, foliicolous fungi, taxonomy

Introduction

Palmer (1994) was the first to report the presence of *Rutstroemia coracina* from the European continent. This species was initially recorded in Algeria and was documented only by a coloured plate as "*Peziza coracina* DR. et Lév." in Durieu (1848: pl. 28 fig. 4) reproduced here as Fig. 1, which depicts apothecia (on a *Quercus ilex* leaf) with egg-shaped spores, paraphyses, and asci. Although no text accompanied the 1848 protologue, the figure caption published in Durieu (1869: 10) identified the substrate as the undersurface of a dried *Q. ilex* leaf and detailed the individual elements included in the figure. Later Saccardo (1889: 237) provided a brief description as *Helotium coracinum,* apparently based on the original figure since no microscopic measurements are given and enquiries by J.T. Palmer with herb. PAD (Università degli Studi di Padova) ascertained that there is no exsiccatum under this epithet in Herb. P.A. Saccardo.

The first authentic description seems to be by Dennis (1964: 39–40, fig. 12), who studied the type collection preserved in PC. Reporting ascospores "11–13 × 5–5.5 µm" and an "[e]xcipulum composed of parallel hyphae with glassy walls," he transferred the species to *Rutstroemia*.

Spooner (1981: 283, 285, fig. 22) redescribed the species from a few apothecia on a *Quercus ilex* leaf collected by M.C. Clark in 1977 near Somerset, southwest England, and recombined it in *Lanzia,* as he failed to find the "glassy walls" found by Dennis. He reported similar spore measurements to those mentioned by Dennis for the type.

In 1992, Palmer visited the Allerford plantation between Bossington and Porlock, Somerset, where Mr. Clark had collected his leaf, and collected large samples of dead *Q. ilex* leaves from different parts of the wood, which subsequently produced apothecia in natural culture. His measurements for the living spores (in tap water) were 12.4–16.8 (18.4) × 4.8–7.6 (8) µm.

Palmer (1994) also restudied the type remnants in PC and found a minute fragment (approximately haft an apothecium) among the stipes of immature apothecia on one of the two spiny leaves of the holotype; although it was not possible to make a detailed study, he managed to draw ovoid spores without any sign of guttulation (measuring 10.6–13.9 × 4.5–6.5 (7.3) µm), one ascus (measuring 106 × 12 µm), and scarce hyphae suspected to be from the gelatinous layer in the ectal excipulum protruding as obtuse "hairs" (3.3–6.0 µm wide) – reproduced here as Fig. 2. In addition, Palmer (1994: 226–228, figs 1–5) made good collections in northern Greece in March 1988, mainly on old, fallen leaves of *Quercus coccifera* and *Q. ilex* on Mount Athos and Mount Olympus but also on a leaf of *Quercus pubescens* (= *Q. humilis*) on Mt Athos.

This species was collected and identified for the first time in Spain by R.G. in 1983 on old, fallen leaves of *Quercus ilex* subsp. *ballota* at Tamajón (Guadalajara province). The identification was later confirmed by Palmer, who had the opportunity to record the species in subsequent years, including collections by Dr. A. Raitviir (Palmer & Galán 1999, Galán 2001). The area is open with scattered trees (intermixed with *Juniperus thurifera*), and leaf litter is sparse. More recently, during a field trip through Guadalajara as part of the XIII Congress of European Mycologists in Alcalá de Henares (September, 1999), several attendees had the opportunity to collect the species in abundance, including Drs. B. Spooner, M.M. Nauta and H.O. Baral, who kindly provided us with herbarium samples. More recent findings of the species by two of us (F.P.G. & A.G.) on old fallen leaves of *Quercus ilex* subsp. *ballota* at Colmenarejo (Madrid province) allow us not only to widen the distribution of *Rutstroemia coracina* in Spain but also to complete its description. In addition, Palmer (pers. comm.) also recorded and identified this species in 1990 on Minorca (Balearic Islands, Spain) growing on *Quercus ilex* leaves.

Fig. 1. Illustration of the type of *Peziza coracina* Durieu & Léveille, Expl. Sci. Algérie, Bot. 1: t. 28, f. 4 (1848).

From the beginning, Palmer (1994) found it noteworthy that the greyish green tinges of apothecia in both freshly collected material or that developed in damp chambers were overlooked in Spanish material, which more closely resembled the reddish brown color in the original Algerian plate. That might suggest the existence of two different taxa, but otherwise all specimens appeared to be microscopically identical.

Since the type material preserved in PC allows no further revision, we here select as epitype a very rich Spanish collection (about 30 fallen leaves of *Quercus ilex* subsp. *ballota,* bearing ca. 60–70 apothecia) whose habit, colour, and microscopic features (mainly ascospore shape and size) match the holotype very well.

There has been continued controversy since Dennis (1964) reported an "excipulum composed of parallel hyphae with glassy walls." This was why the taxon was first combined in *Rutstroemia* and why Spooner (1981) transferred the species to *Lanzia,* remarking, "I have not been able to find glassy walls." Spooner later had opportunity to re examine one of the Greek collections (J.T.P. 4418) identified as *R. coracina* and agreed entirely with Palmer's identification. He noted the presence of gelatinized tissue, which made him review the British material where he found "a gelatinous matrix to the ectal hyphae, something which I overlooked when referring the species to *Lanzia*" (B.M. Spooner, in letter). The microtome sections (Figs 6, 7) of English, Greek, and Spanish material show a clear gel layer in the ectal excipulum, although the thickness is not as prominent as in other *Rutstroemia* spp. and also is somewhat variable in width from collection to collection. Nonetheless, we feel that the species was correctly placed in *Rutstroemia* by Dennis (1964).

Rutstroemia coracina appears to be known only from Algeria (where the actual type locality is unknown), Britain (Spooner 1981), Greece (Palmer 1994), France (Palmer, pers. comm.), Turkey [K(M) 49709 Spooner, pers. comm.], and Spain.

Materials & methods

Measurements of living cells (marked *) were made in tap water or IKI (1% Lugol's solution), with dead cells (marked †) in tap water, Melzer's reagent (MLZ), KOH 2%, and aqueous cresyl blue (CRB) 0.5%. Line drawings were made using a Nikon Labophot-2 either by hand without the aid of a camera lucida (C.E.H.) or with a drawing tube incorporated into the light microscope (R.G.) with magnifications up to 1250×. Photomicrographs were made with using a Nikon Labophot-2 equipped with phase contrast and camera. Macroscopic pictures of fructifications in fresh state showing the general habit were taken in situ (by F.P.G.) or at the lab (by C.E.H.). Apothecial colours are cited according to Locquin (1985). Specimens have been deposited in AH and J.T.P. (personal herbarium of the late Mr. J. Terence Palmer, currently deposited in CUP) with additional specimens in H.O.B. (personal herbarium of Hans O. Baral, Tübingen, Germany) and K.

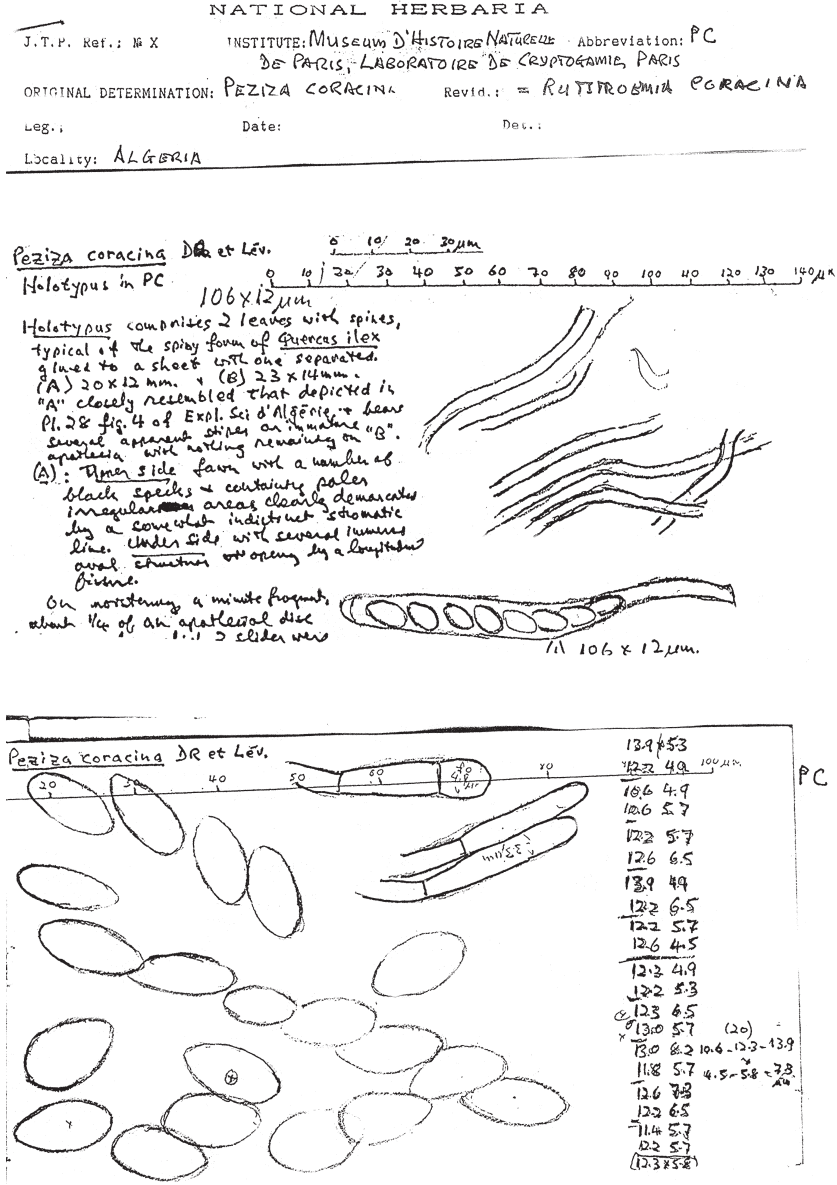

NATIONAL HERBARIA
J.T.P. Ref.: № X
INSTITUTE: Museum D'Histoire Naturelle de Paris, Laboratoire de Cryptogamie, Paris
Abbreviation: PC
ORIGINAL DETERMINATION: Peziza coracina
Revid.: = Rutstroemia coracina
Leg.:
Date:
Det.:
Locality: Algeria
Peziza coracina DR et Lév.
Holotypus in PC
106 x 12 µm
Holotypus comprises 2 leaves with spikes, typical of the spiny form of Quercus ilex glued to a sheet with one separated. (A) 20 x 12 mm. + (B) 23 x 14 mm. "A" closely resembled that depicted in Pl. 28 fig. 4 of Expl. Sci d'Algérie. It bears several apparent stipes or immature "B". apothecia with nothing remaining on "B".
(A): Upper side fawn with a number of black specks & contiguity paler irregular areas clearly demarcated by a somewhat indistinct schematic line. Under side with several immature oval structures or opening by a longitudinal fissure.
On rotating a minute fragment about 1/4 of an apothecial disc
1/1 3 slides were
1/1 106 x 12 µm.
Peziza coracina DR et Lév.
PC

Fig. 2. *Rutstroemia coracina* (holotype, PC): Annotations and drawings by J.T. Palmer showing excipular hyphae and an ascus (up), ascospores, and "hyphoid" hairs (down).

Fig. 3. *Rutstroemia coracina* (AH 7641, epitype) growing in natural habitat.
Photo F.P.G. in situ (bar = 10 mm).

Fig. 4. Apothecia of *Rutstroemia coracina* (C.E.H. 08468) under high magnification.
Photo C.E.H. ex situ (bar = 1 mm).

Taxonomy

Rutstroemia coracina (Dur. & Lév.) Dennis, Persoonia 3: 39 (1964) FIGS. 1–7

≡ *Peziza coracina* Dur. & Lév., Expl. Sci. Algérie, Bot. 1: t. 28, f. 4 (1848)

≡ *Helotium coracinum* (Dur. & Lév.) Sacc., Syll. Fung. 8: 237 (1889)

≡ *Calycina coracina* (Dur. & Lév.) Kuntze, Revis. Gen. Pl. 3(3): 448 (1898)

≡ *Ciboria coracina* (Dur. & Lév.) Boud., Hist. Class. Discom. Eur.: 106 (1907)

≡ *Lanzia coracina* (Dur. & Lév.) Spooner, Trans. Br. Mycol. Soc. 76: 283 (1981)

TYPE: Algeria (**Holotype**, PC). Spain, Madrid, Colmenarejo, UTM 30TVK1489, 885 m a.s.l., on petioles, lower (more exceptionally upper) faces and veins of fallen leaves of *Quercus ilex* subsp. *ballota,* 27.XI.2011, Prieto & González, det. R.G. , rev. C.E.H. (**Epitype designated here**, AH 7641).

APOTHECIA superficial, scattered, cupulate, stipitate, castaneus to reddish-brown, merging from darkened areas of the substrate. DISC slightly concave becoming plane, flesh colour (i.e., russus B4f) to yellowish-olivaceous (i.e., venetus S2d), tending to darken with age, 0.5–2.0 mm in diameter. RECEPTACLE cupulate, covered with a sparse and irregular net of brown fibrils, vertically arranged with adpressed ends, sometimes protruding and giving a fringed appearance, colour similar to or concolorous with disc and stipe, sometimes chestnut (with varying reddish brown shades). MARGIN ochre, chestnut to reddish-brown, crenulate. STIPE equal in length to or longer than disc diameter, reddish-brown often almost black at the base, 0.3–3.0(–6.0) mm long. ECTAL EXCIPULUM composed of three layers: outer zone, 12–38 µm thick, formed of some sparse rows of loosely branched, flexuous, brown hyphae (3.7–5.5 µm in diam. †) arranged parallel to the surface, with thin walls encrusted with pigment giving a typically banded aspect and protruding as agglutinated "hairs" on flank and stipe or in groups as "clavate hairs" at the margin to form teeth; the medial layer of textura oblita, 15–60 µm thick, formed of flexuose, subhyaline to light pale ochre hyphae, arranged parallel or at a low angle to the surface, with very narrow lumina and strongly gelatinized walls; the inner zone of imprecise thickness, formed of prismatic to doliform cells (textura prismatica) and not well delimited from the medullary excipulum, hyphae 5–10 µm (†) in diameter, with pale chestnut content and thin walls. MEDULLARY EXCIPULUM well developed, of textura intricata and subhyaline cells with thin, smooth walls (3–5 µm in diam. †). SUBHYMENIUM compact, poorly developed and indistinguishable from the medullary area, apart from the brownish colour and highly intricate texture. ASCI 8-spored, cylindric-clavate, tapering at base to form a slender stalk, emerging from croziers, a round, truncate to fissured apex, with a long pore outlined J+ (deep blue) in both IKI and Melzer's reagent, 125–152 ×11–13 µm (*), 110–130(–140) ×9–11 µm (†). SPORES biseriate to uniseriate or irregularly biseriate (in dead state), hyaline, ellipsoid to elliptic-

fusoid, slightly inequilateral, regularly unicellular, rarely one to three-septate when over-mature, exceptionally producing subglobose microconidia (2–2.5 µm * diam.) at one or both apices, with high number of globose lipid bodies (1–2 µm) occupying most of the cytoplasm in the living spores (multiguttulate), forming two large distinct apical drops in dead state by coalescence, measuring 14–17(–18) × 6–7.5 µm (*), 12–15(–16.8) × (5–)5.5–6.8(–7.2) µm (†). PARAPHYSES straight, filiform to subclavate ca. 2–2.5 µm diam. (*), enlarged to 4–5.5 µm (*) at the apex, sparsely septate, simple or branched below, equaling or scarcely exceeding the asci in length, bearing brown to reddish-brown vacuolar contents ("refractive vacuolar bodies" sensu Baral 1985) in the living state.

ADDITIONAL SPECIMENS EXAMINED: **SPAIN**, **GUADALAJARA**, **Tamajón**, surroundings of Ermita del Humilladero, on a fallen leaf of *Quercus ilex* subsp. *ballota* in an open mixed wood (with *Juniperus thurifera*) with scattered trees and leaf litter sparse on the ground, 18.XI.1983, Galán, Moreno & Checa, det. R.G., rev. Palmer (AH 6235; duplicate, J.T.P. as permanent slides); on stromatized petioles and veins of fallen old *Quercus ilex* subsp. *ballota* leaves, 3.XII.1984, Galán, Moreno & Palmer, det. J.T.P. (J.T.P. 4125); 3.XII.1991, Galán & Raitviir, det. R.G. & J.T.P. (AH 6720; duplicate, J.T.P. 4695). **Cifuentes**, between Oter and Carrascosa de Tajo, on fallen leaves of *Quercus ilex* subsp. *ballota*, 25.IX.1999, Nauta & Spooner, det. J.T.P. (J.T.P. 4978; duplicates, AH 7334, K). **Mirabueno**, surroundings, 1000 m a.s.l., on side and veins of fallen leaves of *Quercus ilex* subsp. *ballota* lying on ground, 24.IX.1999, Baral & Marson, det. Baral, rev. J.T.P. (J.T.P. 4977; duplicates, AH 7335, H.O.B. 6477E). **MADRID**, **Colmenarejo**, UTM 30TVK1489, 885 m a.s.l., on petioles, lower (more exceptionally upper) faces and veins of fallen leaves of *Quercus ilex* subsp. *ballota,* 18.XI.2011, det. R.G. (AH 7640); 11.XII.2011, det. F.P., rev. C.E.H. (C.E.H. 08468). **ENGLAND, SOMERSET,** between **Allerford** and **Bossington**, on old, fallen leaves of *Quercus ilex* sampled, (apothecia developed in damp chamber from 26.IV–30.V.1992), 20.III.1992, Palmer, det. J.T.P., rev. R.G. (J.T.P. 4713; duplicate, AH 6813); (J.T.P. 4714; duplicate, AH 6812). **GREECE, MACEDONIA**, **Athos Mountain**, surroundings of Monastery Iveron, on stromatized fallen leaves of *Quercus ilex* subsp. *ilex*, 25.III.1988, Palmer, det. J.T.P., rev. R.G. (J.T.P. 4415; duplicate, AH 6604).

COMMENTS—There are at least three additional *Rutstroemia* species usually or occasionally reported from decaying *Quercus* leaves: *R. sydowiana* (Rehm) W.L. White (widely distributed at the Mediterranean basin) with "allantoid" spores (nearly ovoid, with basally bent beaks); *R. petiolorum* (Roberge ex Desm.) W.L. White (typically reported on *Fagus* leaves in Europe but occasionally also appearing on *Quercus* leaves) with allantoid to reniform spores with small polar gel caps; and *R. latispora* K.S. Thind & Saini, later transferred to *Lambertella* (Spooner 1987), found on *Quercus incana* leaves in the north-west Himalayas, having asymmetrical and much broader ascospores and longer asci than the two previous species (Thind & Saini 1968). In addition *Rutstroemia "kalevi"* Baral (ined.), which that could be misidentified as *R. coracina* (despite its *Mollisia*-like vacuoles in the paraphyses and narrower spores) grows on stromatized veins and petioles of *Acer* spp. in Central Europe (Baral & Marson 2005).

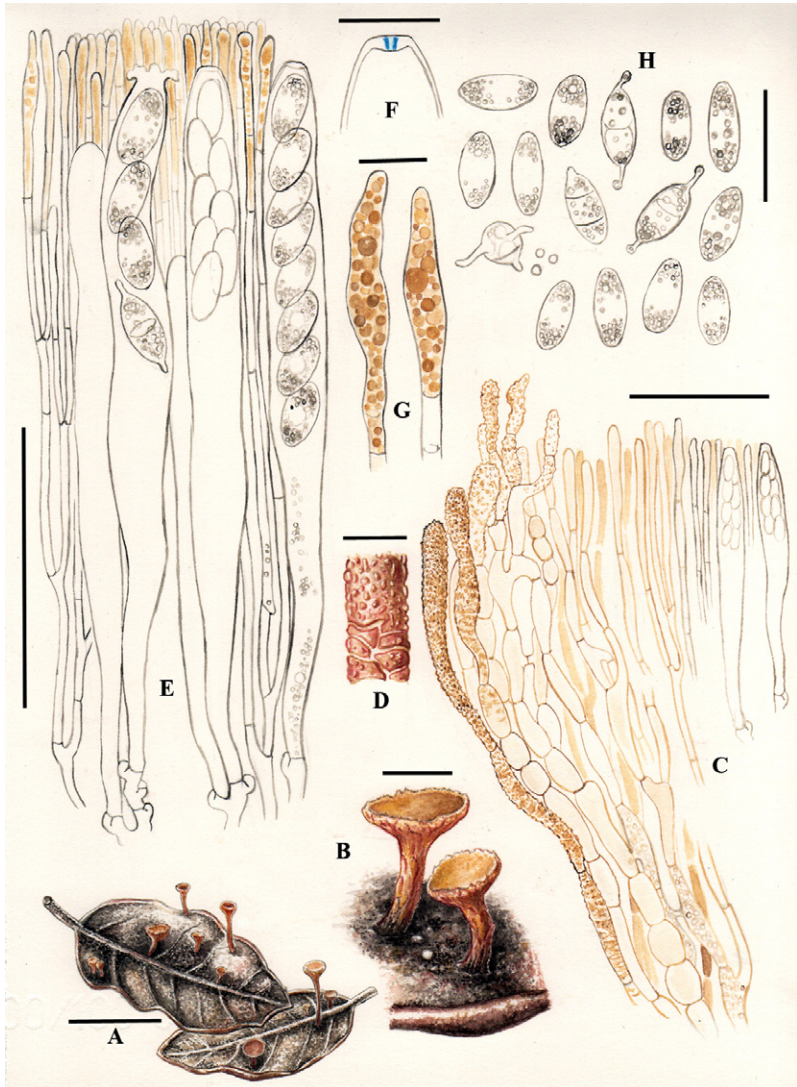
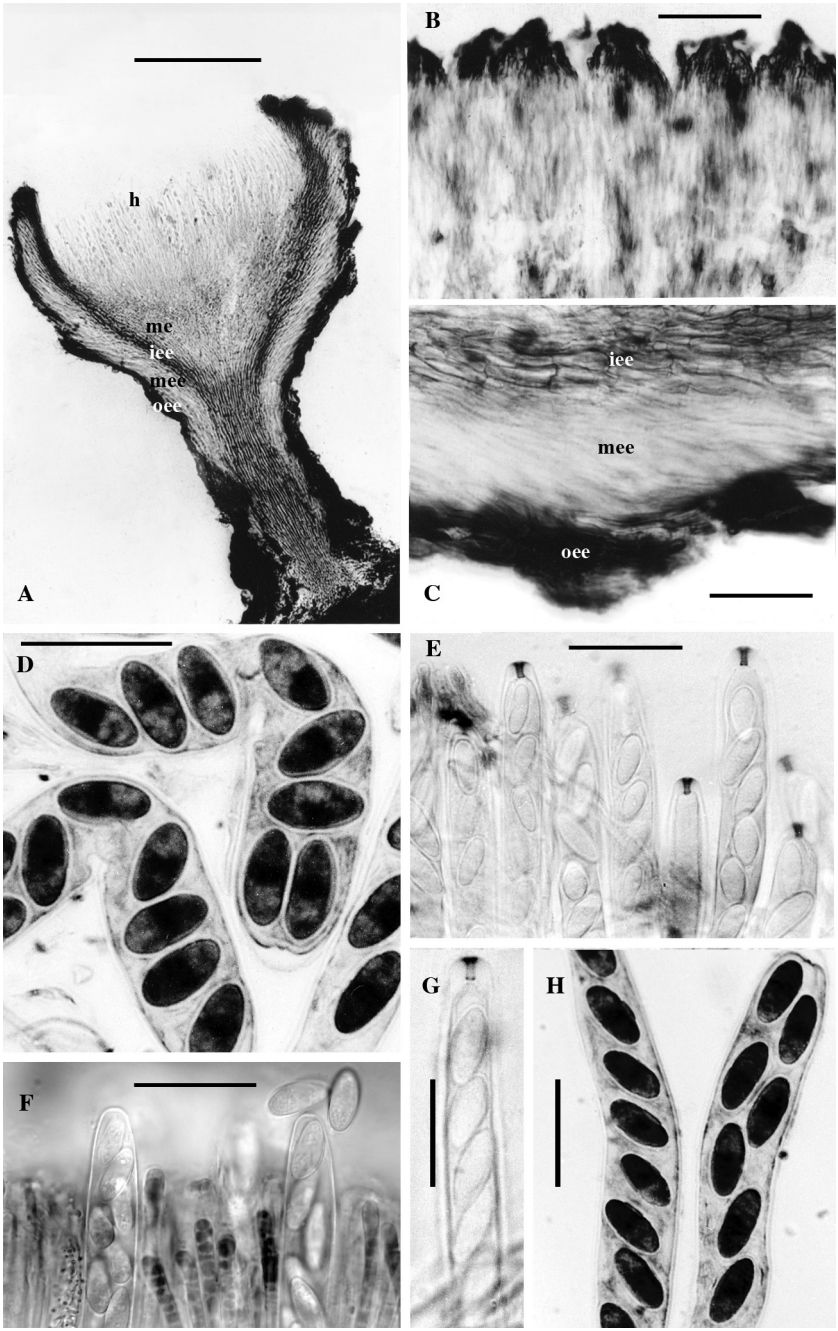


Fig. 5. *Rutstroemia coracina* (AH 7641, epitype): A. Apothecia in their natural habitat (bar = 10 mm); B. Enlarged view of fresh apothecia (bar = 1 mm); C. Cross section of an apothecium (marginal region) (bar = 50 µm); D. Encrusted wall of hyphae of the outer ectal excipulum (bar = 5 µm); E. Hymenium showing asci arising from croziers and paraphyses (bar = 50 µm); F. apex of a mature ascus with an euamyloid ring (bar = 10 µm); G. Paraphyses containing many refractive vacuolar bodies in apical cells (bar = 5 µm); H. Free 0-3-septate ascospores, exceptionally forming small conidia on germ tubes (bar = 20 µm). Mountants: tap water, except fig. F (IKI). Watercolour by C.E.H.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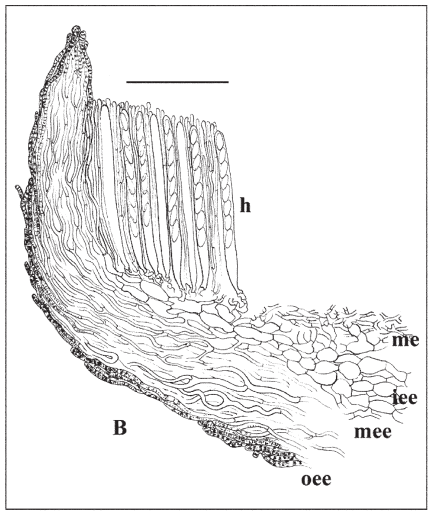

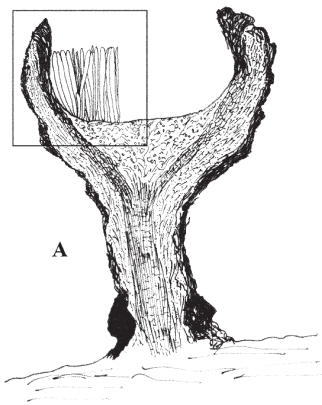

Fig. 6 (left). *Rutstroemia coracina*: A. Radial section of an apothecium (AH 6812; bar = 200 µm); B. Frontal view of the outer ectal excipulum forming marginal teeth (AH 6604; bar = 50 µm); C. Radial section of an apothecium at middle flanks showing a three layered ectal excipulum (AH 6812; bar = 50 µm); D, E, G, H. Asci with ascospores (AH 6235; bar = 20 µm for D, G, H; 25 µm for E); F. Mature asci, free spores and tips of paraphyses (AH 7641; bar = 25 µm). Mountants: 2% KOH (figs. A, B & C); CRB (figs. D & H); MLZ (figs. E & G); tap water (fig. F). Dead state except fig. F. Photographs by R.G. & C.E.H. (fig. F). Abbreviations: outer (= oee), medial(= mee) and inner (= iee) layers of the ectal excipulum; medullary excipulum (= me).

Fig. 7 (above). *Rutstroemia coracina* (AH 6812): A. Radial section of an apothecium (bar = 200 µm); B. Enlarged view of an apothecium (bar = 100 µm), at flank and margin, showing: the outer (= oee), medial(= mee) and inner (= iee) layers of the ectal excipulum; medullary excipulum (= me); hymenium (= h). Dead state (KOH 2%). Drawings by R.G. & C.E.H.

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